Hon. Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Re.: Statement regarding the patent application of Dr. Florian Kern - METHOD FOR IDENTIFYING T-CELL STIMULATING PROTEIN FRAGMENTS, serial No 09/600,564

November 2, 2004

Sir:

I, Rainer P. Woitas, of Bonn, Germany, am a principal investigator and author of the article entitled *CD30 Induction and Cytokine Profiles in Hepatitis C Virus Core-Specific Peripheral Blood T Lymphocytes* that was published in the Journal of Immunology (1997) vol. 159: 1012-1018. Since that time, I have been an author of several research articles and reviews related to cellular immunity.

II,The above-named article describes methods used to identify antigen-specific T cell cytokine profiles induced by peptides from Hepatitis-C Virus (HCV) core protein. When stimulated, T lymphocytes demonstrate increased cellular levels of marker proteins; e.g., the surface protein CD30, interferon-γ and one or more interleukins (e.g., IL-2, IL-4 and IL-10). The increase in marker proteins was quantified by measuring the levels of CD30, interferon-γ and/or various interleukins in comparison to unstimulated T lymphocytes.

1.The method described in the article comprises contacting T lymphocytes with one or more short peptides corresponding to distinct regions of the HCV protein. Peptide-stimulated T lymphocytes were analyzed after immunolabeling of the T lymphocytes with fluorescent antibodies to form fluorescent immune complexes for each protein of interest. The basis of the assay is that the magnitude of the fluorescent signal measured by flow cytometry is proportional to the amount of fluorescent antibody-protein complex present, which in turn, reflects the amount of the protein being measured.

The immunolabeling and quantification of the fluorescent immune complexes was performed after a 40 hour incubation of T lymphocytes with the respective HCV peptides.

2.The peptide stimulation of the T lymphocytes leading to the induction of CD30, interferon--γ and/or interleukins was achieved by incubating the T lymphocytes with 10 µg/ml of synthetic HCV peptides, for 40 hours. This methodology is described in the reference on page 1013, column 2, and the legend of figure 3.

3.Twelve hours prior to the end of the 40 hour peptide-incubation period, the drug monensin was added to the culture medium. The purpose of monensin was merely to decrease the rate of cytokine secretion from the cell, thereby increasing the intracellular concentration of the induced cytokine. The increased intracellular concentration of the cytokines enhances the sensitivity of the fluorescent signal obtained from cytokine-antibody immune complexes.

4.At the end of the 40 hour incubation period with HCV peptides, the T lymphocytes were prepared for flow cytometry by forming the fluorescent immune complexes after fixing the cells in paraformaldehyde and permeablizing the cells with saponin. It is well known that fixation kills the cells; therefore, the fixed and permeabilized cells can only be used for analysis of the fluorescent signal, and cannot be stimulated to any further extent by incubation with the antibodies used to label the cells.

In summary, the immunolabeling of the peptide-contacted T lymphocytes for detecting interferon-γ and/or various interleukins was performed in dead cells with openings in their cell membranes due to saponin treatment.

5.The immunolabeling method is provided in detail on page 1013, col. 2. It is noteworthy that the fixed and permeablized cells were incubated with each of the relevant antibodies for no more than 30 minutes. The length of this time period is clearly much shorter than the 40 hour incubation with the HCV peptides.

It should be emphasized that the 30 minute incubation of antibodies with the fixed permeablized T lymphocytes for flow cytometry is completely unrelated to the 40 hour HCV peptide stimulation of the cells. In addition, the incubation of T lymphocytes with the HCV peptides requires the cells to be alive. Thus,

immunolabeling of dead cells with antibodies cannot exert any effect on the level of peptide-induced stimulation.

Hereby I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. Furthermore, these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both.

Date

Rainer P. Woitas